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AN EFFICIENT SYNTHESIS OF THE CRUSTACEAN HORMONE [12-^{3}H]-METHYL FARNESOATE AND ITS PHOTOLABILE ANALOG [13-^{3}H]-FARNESYL DIAZOMETHYL KETONE

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SUMMARY

Tritium-labelled methyl farnesoate ([^{3}H]-MF, 6.6 Ci/mmol) was prepared from a tritiated allylic alcohol derivative by an efficient new one-pot halogen exchange-reductive dehalogenation sequence. The resulting [^{3}H]-MF was converted in three steps to a diazomethyl ketone analog, [^{3}H]-FDK, which can be used for the photoaffinity labelling of MF binding proteins.

Key words: Methyl farnesoate, crustacean, binding protein, photoaffinity label, farnesyl diazomethyl ketone, diazoketone, reductive dehalogenation, tritium labelling

INTRODUCTION

Methyl farnesoate (MF) was characterized in the hemolymph of adult spider crabs, *Libinia emarginata* (1) and proposed, on the basis of chemical similarity and physiological function, as a *bona fide* crustacean juvenile hormone (2). Earlier, MF had been shown to be an important precursor of JH III biosynthesis in cockroaches (3) and other insects (4). In order to further examine the biochemical role of MF in crustacean physiology, we required ready access to [^{3}H]-MF and to an appropriate photoaffinity-labelled analog.

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Tritium-labelled MF has previously been synthesized from the labelled insect juvenile hormone, [10-^{3}H]-JH III (5) or [12-^{3}H]-JH III (6) by deoxygenation of the 10,11-oxirane. Earlier, [12-^{3}H]-MF had been synthesized by reductive dechlorination of the 12-chlorotrienoate with NaB^3H_4 in dimethyl sulfoxide (DMSO) in the presence of 1,5-hexadiyne (7) but no experimental details were given (8). A tritiated bis homolog of methyl farnesoate of low specific activity was also prepared by this one-step, reductive dehalogenation method albeit in low (40%) yield (9).

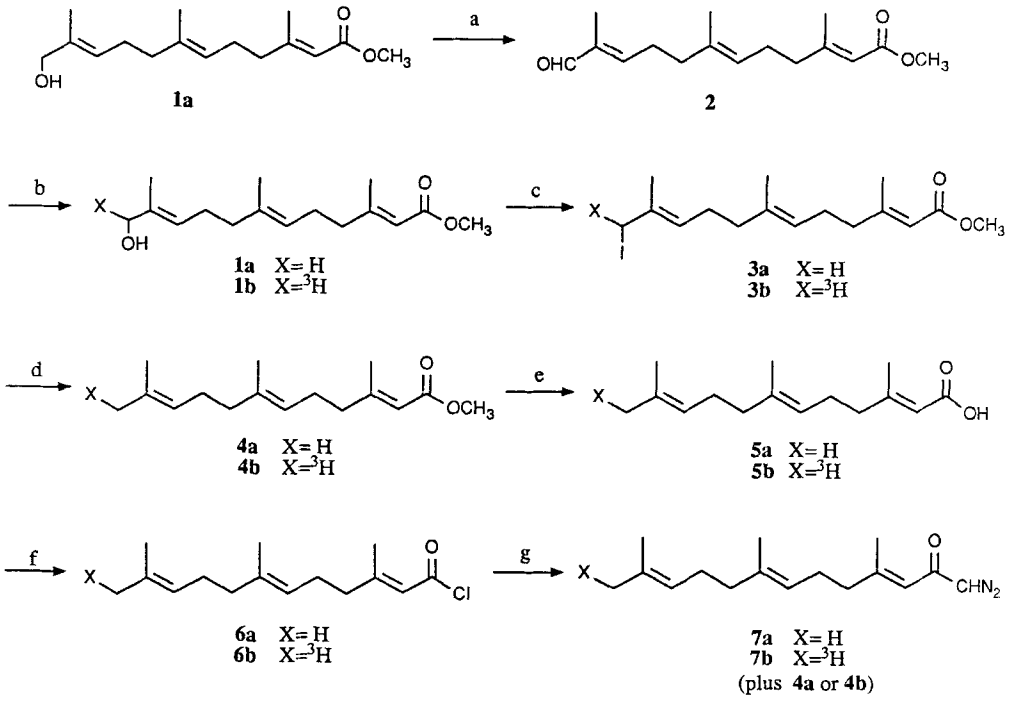
The need for tritium-labelled MF and its photoaffinity analog, farnesyl diazomethyl ketone (FDK) of high specific activity, prompted us to develop a short synthetic route for these labelled compounds. Unlabelled FDK was previously synthesized for labelling studies of insect juvenile hormone binding proteins (10, 11); however, the synthesis reported did not allow ready access to tritium labelled material.

RESULTS AND DISCUSSION

The synthetic procedures are illustrated in Scheme 1. Methyl 12-hydroxy-farnesoate **1a** (12) was oxidized to the corresponding aldehyde **2** using the N-methylmorpholine N-oxide (NMO) and tetra-*n*-propylammonium perruthenate (TPAP) oxidant system (13). Aldehyde **2** was then reduced with either $NaBH_4$ or NaB^3H_4 to give the corresponding alcohols **1a** or **1b**, respectively. The procedures which follow were essentially identical for the labelled and unlabelled materials except as indicated.

Reductive dehalogenation of alcohol **1** can then conveniently be carried out in one-pot. First, the alcohol **1** was converted into the corresponding iodo derivative **3** using freshly purified (14) methyl(triphenoxy)phosphonium iodide (15) in HMPA. Purification of this reagent was required, since the use of the dark-colored phosphonium salt as received gave incomplete conversion of alcohol **1a** to the intermediate iodide **3a** even in the presence of a large excess of phosphonium salt. The allylic iodide formed was then reduced (16) with $NaBH_3CN$ to give **4a** in 62% yield. For the labelled material, reductive dehalogenation was achieved with potassium 9-cyano-9-hydrido-9-borabicyclo[3.3.1]nonane (9-BBNCN) to afford **4b** in 97% overall yield.

The farnesoic acids **5** were then obtained by saponification with NaOH in aqueous ethanol. Treatment of the acids **5** with excess of oxalyl chloride in benzene



SCHEME 1. Radiosynthesis of [12-^{3}H] methyl farnesoate and its diazomethyl ketone analog.

Reagents: (a) TPAP, NMO, CH_2Cl_2; (b) $NaBH_4$ or NaB^3H_4, ethanol; (c) $CH_3(PhO)_3P^+I^-$, HMPA; (d) $NaBH_3CN$ or K-9-BBNCN, HMPA; (e) NaOH, aq. EtOH; (f) $(COCl)_2$, benzene; (g) CH_2N_2, CH_2Cl_2, ether .

followed by removal of the solvent, dissolution of the acid chlorides **6** in CH_2Cl_2 and addition of excess ethereal diazomethane gave a mixture of diazoketones **7** and methyl esters **4** which were then easily separated by flash chromatography and HPLC. Both [^{3}H]-MF (**4b**) and [^{3}H]-FDK (**7b**) had specific activities of 6.6 Ci/mmol. Experiments using [^{3}H]-FDK to photoaffinity label specific MF-binding proteins in crustaceans will be described elsewhere (17).

MATERIALS AND METHODS

All reactions were performed under N_2. [^{3}H]-Sodium borohydride (26.4 Ci/mmol) was purchased from Amersham. CH_2Cl_2 and hexamethylphosphoramide (HMPA) were freshly distilled over CaH_2. Benzene was distilled over sodium metal. Flash chromatography purifications were performed on Woelm Silica (32-63 μm) in disposable Pasteur pipets.

^{1}H-NMR spectra were determined on a General Electric QE-300 spectrometer. IR spectra were recorded on a Perkin Elmer 1600 Series FTIR spectrometer. UV spectra were measured in hexane on a Perkin Elmer Lambda 5 UV/VIS spectrophotometer. The HPLC analyses and preparative separations were carried out on a Waters Associates chromatograph, fitted with a variable-wavelength Kratos-Schoeffel SF 770 Spectroflow detector, using a Fisher Resolvex Sil (4.6 mm x 25 cm) column. The radiochemical homogeneity of the labelled compounds was assessed by TLC using EnHance spray and fluorography using Kodak XAR-5 film. Radioactive samples were counted in an LKB 1218 RackBeta liquid scintillation counter using ScintiVerse II (Fisher Scientific) scintillation cocktail. The tritiated compounds were stored in toluene-heptane solution below -15 °C in sealed ampoules.

Methyl (2*E*,4*E*)-12-Oxofarnesoate (2). A mixture of 32.5 mg (0.122 mmol) of methyl 12-hydroxyfarnesoate (**1a**) (12, 18), 100 mg of crushed 4Å molecular sieves (Aldrich), 22 mg (0.187 mmol) of NMO, and 3 mL dry CH_2Cl_2 was stirred at room temperature for 10 min. Then, 3 mg (0.009 mmol) of TPAP was introduced and the stirring was continued for 3 h at room temperature. The reaction mixture was then diluted with 10 mL of hexane, 25 mg of Na_2SO_3 and 100 mL of water were added. After stirring for 10 min, the mixture was dried ($MgSO_4$) and filtered through a short pad of Celite. The filtrate was concentrated and the residue was purified by flash chromatography (2% EtOAc in hexane) to give 24 mg (74% yield) of pure aldehyde **2**. ^{1}H-NMR ($CDCl_3$): δ 1.62 (3H, s), 1.72 (3H, s), 2.1 (9H, m), 2.42 (2H, q, J = 7 Hz), 3.66 (3H, s), 5.11 (1H, s), 5.64 (1H, s), 6.43 (1H, t, J = 7 Hz), 9.36 (1H, s).

Methyl (2*E*,4*E*)-12-Hydroxyfarnesoate (1a). About 1 mg (26 mmol) of $NaBH_4$ was added to a solution of 20 mg (76 mmol) of aldehyde **2** in 0.5 mL of ethanol at 0 °C. The reaction mixture was stirred at 0-5 °C for 30 min and at room temperature for 1 h. Then 4 mL of 10% EtOAc in hexane and 0.3 mL of 1 N NaH_2PO_4 was added to the reaction mixture, the phases were separated, the aqueous layer was extracted with 10% EtOAc in hexane (2 x 1 mL), and the organic extracts were combined, washed with water and brine, dried ($MgSO_4$), and concentrated. Purification by flash chromatography (10% EtOAc in hexane) gave 18 mg (89% yield) of alcohol **1a**.

^{1}H-NMR ($CDCl_3$): δ 1.59 (3H, s), 1.65 (3H, s), 1.8-2.3 (8H, m), 2.16 (3H, s), 3.68 (3H, s), 3.97 (2H, s), 5.08 (1H, bs), 5.36 (1H, t, *J* = 7 Hz), 5.66 (1H, s).

Methyl (2*E*,4*E*)-Farnesoate (4a). A solution of 7.2 mg (0.027 mmol) of alcohol **1a** and 26 mg (0.058 mmol) of methyl(triphenoxy)phosphonium iodide, previously washed with EtOAc and dried *in vacuo*, in 500 µL of dry HMPA was stirred at room temperature for 2 h. Then 9.0 mg (0.143 mmol) of $NaBH_3CN$ was added and the stirring was continued for 2 h at room temperature. The reaction mixture was diluted with 8 mL of pentane, 0.5 mL of phosphate buffer (pH = 5), and 1 mL of water, and the phases were separated. The aqueous layer was extracted with pentane (3 x 5 mL), the organic layers were combined, washed with water and brine, dried ($MgSO_4$), concentrated and purified by flash chromatography (5% EtOAc in hexane) to give 4.2 mg (62% yield) of pure product **4a**. ^{1}H-NMR ($CDCl_3$): δ 1.59 (6H, s), 1.67 (3H, s), 1.99 (2H, t, *J* = 6 Hz), 2.04 (2H, t, *J* = 6 Hz), 2.15 (3H, s), 2.20 (4H, m), 3.64 (3H, s), 5.09 (2H, bs), 5.67 (1H, s).

(2*E*,4*E*)-Farnesoic Acid (5a). A mixture of 3.5 mg (0.014 mmol) of ester **4a** and 0.5 mL of 0.5 N NaOH solution in 1:1 water-ethanol was stirred at 40 °C for 4 h. Then, half of the solvent was evaporated under N_2, 0.5 mL of water and 2 mL of hexane were added, the mixture was placed in an ice bath and acidified with about 100 µL of 5 N HCl solution. The phases were separated, the aqueous layer was extracted with 20% ethyl ether in hexane (3 x 2 mL), and the organic extracts were combined, dried ($MgSO_4$), concentrated and purified by flash chromatography (15% EtOAc in hexane) to give 2.8 mg (85% yield) of acid **5a**. ^{1}H-NMR ($CDCl_3$): δ 1.59 (6H, s), 1.67 (3H, s), 2.00 (2H, t, *J* = 6 Hz), 2.04 (2H, t, *J* = 6 Hz), 2.18 (3H, d, *J* = 1 Hz), 2.21 (4H, m), 5.10 (2H, bs), 5.71 (1H, bs), 11.5 (bs).

(2*E*,4*E*)-Farnesyl Diazomethyl Ketone (7a). A solution of 2.0 mg (0.008 mmol) farnesoic acid **5a**, 0.25 mL of dry benzene and 45 µL (0.045 mmol) of a 1 M oxalyl chloride solution in benzene was stirred at room temperature for 1.5 h, then at 35-40 °C for an additional 1 h. Then the solution was concentrated *in vacuo*, 0.1 mL of dry benzene was added and the solution was concentrated again. The resulting crude acid chloride was then dissolved in 0.2 mL of dry CH_2Cl_2, cooled to -10 °C, and 0.15 mL (about 15-fold

excess) of an approximately 1 M ethereal diazomethane solution (19), previously dried over KOH pellets at 0-5 °C for 3 h, was added. After stirring at -5 °C for 2 h and at 10-15 °C for 1 h, an additional amount of 0.1 mL of diazomethane solution was added to the reaction mixture and the stirring was continued at room temperature for 3 h. The solution was then concentrated *in vacuo* and the residue was purified by flash chromatography (5% EtOAc in hexane) to give ca. 1 mg (46 % yield) of diazomethyl ketone **7a** and ca. 1 mg (50 % yield) of methyl farnesoate **4a**. IR of **7a** (CCl_4): 2100, 1645, 1615, 1450, 1390,1145,, 1100 cm^{-1}. ^{1}H-NMR ($CDCl_3$) of **7a**: δ 1.59 (6H, s), 1.67 (3H, s), 1.99 (2H, t, J = 6 Hz), 2.04 (2H, t, J = 6 Hz), 2.14 (3H, s), 2.20 (4H, m), 5.07 (2H, bs), 5.17 (1H, s), 5.74 (1H, s). Extinction coefficient (hexane): ε_{259} = 14,400.

Methyl [12-^{3}H]-(2*E*,4*E*)-12-Hydroxyfarnesoate (1b). Three hundred mCi (11.4 µmol) of NaB^3H_4 (26.4 Ci/mmol), dissolved in 0.50 mL of cold ethanol containing 50 µL of 0.01 N aq. NaOH solution, was added to a solution of 18 mg (68 µmol) of aldehyde **2** in 0.35 mL of ethanol at 0 °C. The reaction mixture was stirred at 0-5 °C for 40 min, then at room temperature for 40 min, diluted with 3 mL of 10% EtOAc in hexane solution, and finally quenched with 0.10 mL of 1 N NaH_2PO_4. After stirring for 5 min, the phases were separated, the aqueous layer was extracted with 10% EtOAc in hexane (2 mL), the organic phases were combined, dried ($MgSO_4$), concentrated, and the residue was purified by flash chromatography (5-20% EtOAc in hexane) to give 12.2 mg (98% chemical yield, based on NaB^3H_4) of alcohol **1b**. The radioactivity of the product was 300 mCi (100% radiochemical yield); thus the specific activity of **1b** is 6.6 Ci/mmol.

Methyl [12-^{3}H]-(2*E*,4*E*)-Farnesoate (4b). A solution of 12.0 mg (295 mCi; 44.7 µmol) of alcohol **1b**, 48 mg (106 µmol) freshly purified methyl(triphenoxy)-phosphonium iodide and 0.80 mL of dry HMPA was stirred at room temperature for 3 h. Then, a solution of 45 mg (238 µmol) potassium 9-BBNCN in 0.25 mL dry HMPA was introduced and the reaction mixture was stirred at room temperature for 3.5 h. The reaction mixture was then diluted with 3 mL of hexane, 0.35 mL of 1 M NaH_2PO_4 was added, the phases were separated, the aqueous layer extracted with hexane (4 x 2 mL), the organic extracts were combined, washed with water and brine (2-2 mL), dried

($MgSO_4$), concentrated and purified by flash chromatography (0-10% EtOAc in hexane) to give 10.9 mg (97% chemical yield) of product **4b** of greater than 98% purity as shown by HPLC (hexane) and fluorography. Radiochemical yield: 288 mCi (98%). Specific activity: 6.6 Ci/mmol.

[12-^{3}H]-(2*E*,4*E*)-Farnesoic Acid (5b). Seventy mCi (10.6 μmol) of ester **4b** and 0.35 mL of a 0.5 M ethanol-water (1:1) NaOH solution was stirred at room temperature for 3 h, then at 45-50 °C for 3 h. The reaction mixture was concentrated to its half volume, 0.2 mL of water and 1.5 mL of 15% EtOAc in hexane solution were then added, and the mixture was cooled to 0 °C and acidified with 50 μL of conc. HCl. The phases were separated, the aqueous layer extracted with hexane (3 x 0.5 mL), the organic extracts were combined, dried ($MgSO_4$), concentrated and purified by flash chromatography (5-25% ethyl ether in hexane) to give 64.5 mCi of acid **5b** (92% radiochemical yield). Samples for bioassays were purified by HPLC (13% ethyl ether in hexane containing 0.03% acetonitrile; detector: 217 nm).

[13-^{3}H]-(2*E*,4*E*)-Farnesyl Diazomethyl Ketone (7b). The tritiated farnesoyl chloride (**6b**) was prepared from 25 mCi (3.7 μmol) of acid **5b** in 0.2 mL of dry benzene using 20 μL (20 μmol) of a 1 M oxalyl chloride solution in benzene as described above for the unlabelled acid chloride **6a**. The crude **6b** was then dissolved in 0.2 mL of dry CH_2Cl_2 and treated with a large excess (about 100-fold) of 1 M dry diazomethane solution in ether as described above for **6a**. The resultant crude reaction mixture was then first purified by flash chromatography (5-15% EtOAc in hexane) to give 18 mCi (72% radiochemical yield) of methyl [12-^{3}H]-farnesoate (**4b**) and 6 mCi (24% radiochemical yield) of tritiated diazomethyl ketone **7b**. For bioassays, this material was repurified by HPLC (9% ethyl ether in hexane containing 0.03% of acetonitrile; detector: 250 nm) to give 4.8 mCi of a greater than 98% pure (HPLC) diazomethyl ketone **7b**.

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